A biomimetic extraction for measuring the bioaccessibility of PAH to benthic invertebrates

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Sediment Extraction Methods

Analysis for organic compounds

Extract with strong organic solvent like dichloromethane, hexane, etc.

Contaminated Sediment

Deposit-feeding animal

"Extractant" is gut fluid, typically with neutral pH Analysis for metals

Extract with strong acid like nitric and/or hydrochloric

Comparative Extraction Efficiency

Extraction of benzo(a)pyrene from sediment

Acetonitrile

100%

Worm gut fluid

22%

Extraction of zinc from sediment

4 N Hydrochloric acid

98%

Worm gut fluid

14%

Arenicola brasiliensis



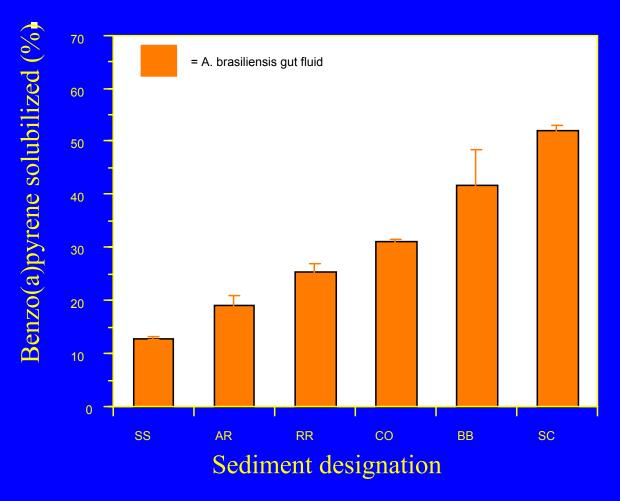
Gut of A. brasiliensis



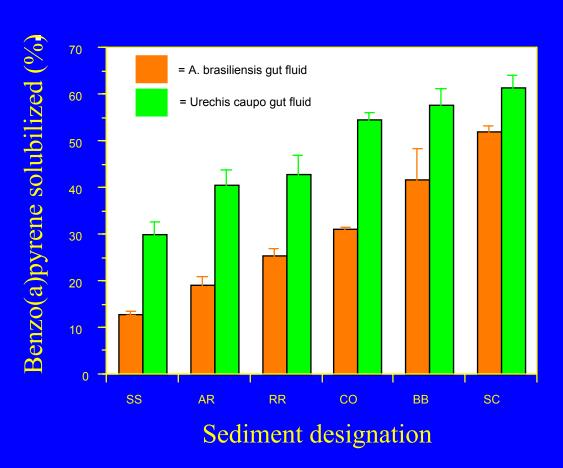
Procedure for digestive fluid extraction of sediments

- 1. Obtain gut fluid from midgut of large deposit feeder.
- 2. Composite fluid from multiple individuals.
- 3. Store fluid at -90 C
- 4. Mix 0.5 g sediment with 0.8 ml fluid.
- 5. Continuous agitation for 1-2 hr.
- 6. Centrifuge to recover supernatant.
- 7. Amount of contaminant solubilized is considered to represent the fraction bioaccessible to the organism through the digestive route.

Comparative bioaccessibility among sediments



Comparative bioaccessibility among sediments



Is the approach useful to predict in vivo bioaccessibility or bioavailability?

If digestive fluid extraction is to be used to assess bioaccessibility of contaminants from sediments....

What is the mechanism for enhanced solubilization in gut fluid?

Species from which gut fluid was obtained

Annelida

Abarenicola pacifica

Abarenicola vagabunda

Arenicola brasiliensis

Arenicola marina

Nephtys discors

Travisia foetida

Mollusca

Archidoris montereyensis Katharina tunicata

Anthozoa

Urticina crassicornis

Echinodermata

Brisaster latifrons

Chirdota sp.

Eupentacta quinquesimata

Molpadia intermedia

Parastichopus californicus

Echiura

Echiurus echiurus

Urechis caupo

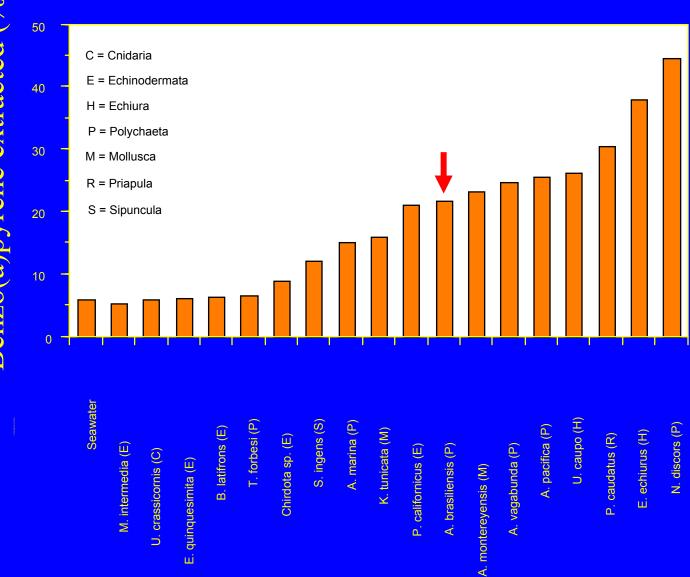
Sipuncula

Siphonosoma ingens

Priapula

Priapulus caudatus

Benzo(a)pyrene extracted (%)

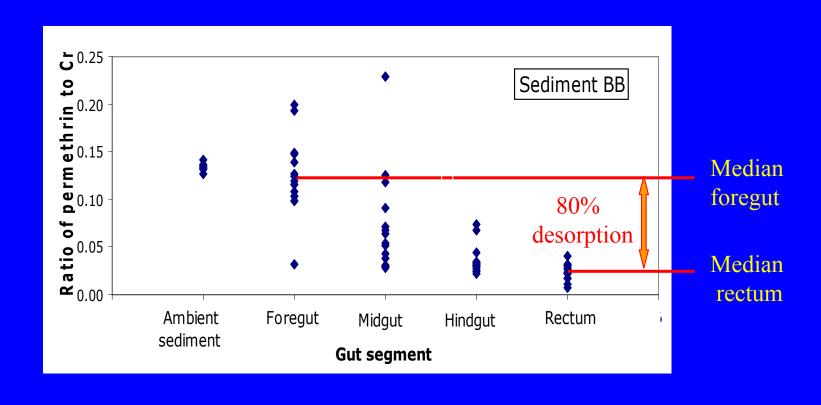


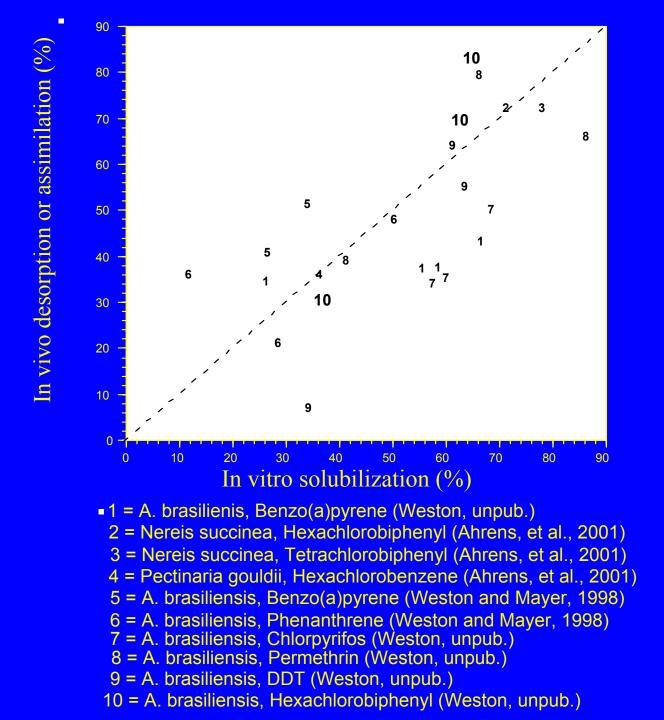
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Approach to measure in vivo bioaccessibility





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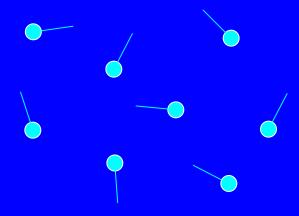
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Basic properties of surfactants

Below Critical Micelle Conc.

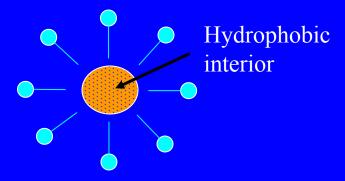
Surfactant molecules unorganized



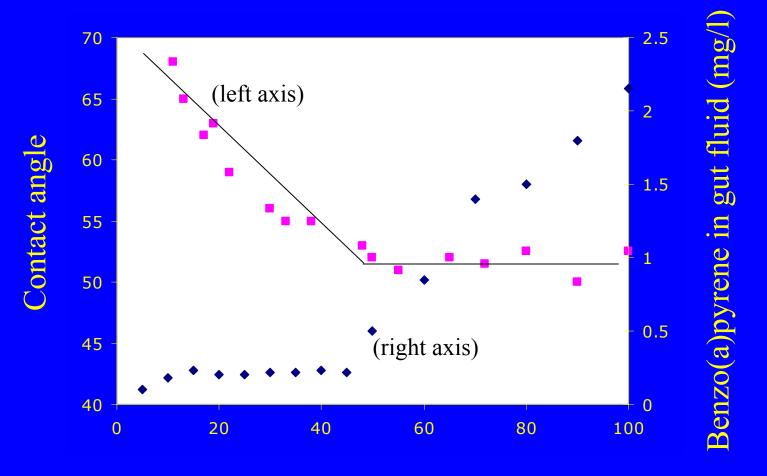
Surface tension inversely related to surfactant concentration

Above Critical Micelle Conc.

Surfactant molecules in micelles



Surface tension constant and independent of micelle conc.



Percent gut fluid (in seawater)

Data from Voparil and Mayer (2000)

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Advantages of a synthetic gut fluid

- Greater availability to investigators without access to a suitable gut fluid "donor" species.
- Makes possible use of greater fluid quantities, lessening risk of ligand saturation during extraction.
- Avoids differences in gut fluid properties between individuals, times of collection, populations, etc.
- Low initial contaminant concentration.

Using a sodium taurocholate and bovine serum albumin gut fluid mimic...

- PAH solubilization potency of the artificial cocktail correlated with <u>Arenicola</u> gut fluid with an r-squared of 0.84.
- In tests with 12 PAH in 4 field-contaminated sediments, PAH solubilization by the cocktail was within a factor of 2 of <u>Arenicola</u> gut fluid in 40 of 48 PAH-sediment combinations.

Data from Voparil and Mayer (2004)

Summary

- 1. Digestive fluid extraction provides a biologically relevant and rapid assay of hydrophobic contaminant bioaccessibility.
- 2. The technique shows very good ability to predict in vivo bioaccessibility for deposit feeders. Predicting bioaccumulation is a taller order, and is probably especially difficult for metals.
- 3. A cocktail that is mechanistically similar to gut fluid shows promise and permits broad utilization of the technique to quantify bioaccessibility.